PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and
Trademark Office
Washington, D.C.

in its capacity as elected Office

Date of mailing: 14 October 1993 (14.10.93)	
International application No.: PCT/GB93/00586	Applicant's or agent's file reference: M92/0120/PCT
International filing date: 22 March 1993 (22.03.93)	Priority date: 28 March 1992 (28.03.92)
Applicant: THE VICTORIA UNIVERSITY OF MANCHESTER et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International preliminary Examining Authority on:

29 March 1993 (29.03.93)

[ ] in a notice effecting later election filed with the International Bureau on:

_____

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer:

J. Zahra

Telephone No.: (41-22) 730.91.11

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING
DOCUMENT TRANSMITTED

To:

United States Patent and Trademark
Office
Washington, D.C.

in its capacity as elected Office

Date of mailing:
04 March 1994 (04.03.94)

International application No.:
PCT/GB93/00586

International filing date:
22 March 1993 (22.03.93)

Applicant:
THE VICTORIA UNIVERSITY OF MANCHESTER et al

The International Bureau transmits herewith the following documents and number thereof:

_____ copy of the international preliminary examination report and annexes (Article 36(3)(a))

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorised officer:

M. Abidine

Telephone No.: (41-22) 730.91.11

PATENT COOPERATION TREATY
PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference **M92/0120/PCT**	**For Further Action** See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International Application No. **PCT/GB 93/00586**	International Filing Date *(day/month/year)* **22 March 1993**	Priority Date *(day/month/year)* **28 March 1992**
International Patent Classification (IPC) **IPC5: A61K 37/02, 39/395; C07K 15/00**		
Applicant **THE VICTORIA UNIVERSITY OF MANCHESTER et al**		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This **REPORT** consists of a total of **5** sheets.

   ☒ This report is also accompanied by **ANNEXES** i.e., sheets of the description, claims and/or drawings amended during international preliminary examination and/or containing rectifications made before this Authority.

   These annexes consist of a total of **4** sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

Date of submission of the demand **22 March 1993**	Date of completion of this report **24 February 1993**
Name and mailing address of the IPEA **The Patent Office, Cardiff Road, NEWPORT, Gwent, NP9 1RH**	Authorized Officer **C Sherrington**
Facsimile No **0633 814444**	Telephone No **0633 814965**

INTERNATIONAL PRELIMINARY [redacted]MINATION REPORT

International [redacted]lication No
PCT/GB 93/00586

I. Basis of the report

1. This report has been drawn on the basis of:

☐ the international application as originally filed.

☒ the description, pages 1-17, as originally filed,
pages, filed with the demand,
pages, filed with the letter of
pages, filed with the letter of

☒ the claims, pages, as originally filed,
pages, as amended under Article 19,
pages, filed with the demand,
pages 18-21, filed with the letter of 21 February 1994
pages, filed with the letter of

☐ the drawings, sheets, as originally filed,
sheets, filed with the demand,
sheets, filed with the letter of
sheets, filed with the letter of

2. The amendments have resulted in the cancellation of: pages:
sheets of drawings No:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box.

4. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

☐ copy of the earlier application whose priority has been claimed.

☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application,

☒ claims Nos. 20

because:

☒ the said international application, or the said claims Nos. 20 relate to the following subject matter which does not require an international preliminary examination *(specify)*:

Method of treatment by therapy of the human or animal body - PCT rule 67.1(iv).

☐ the description, claims or drawings (indicate particular elements below) or said claims Nos . are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims or said claims Nos . are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for said claims Nos .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	claims 1 to 19	YES
	claims	NO
Inventive Step (IS)	claims 1 to 19	YES
	claims	NO
Industrial Applicability (IA)	claims 1 to 19	YES
	claims	NO

2. CITATIONS AND EXPLANATIONS

None of the cited documents either specifically disclose, or, taken together or separately, are considered to lead to, the wound healing compositions, which comprise at least one non-fibrotic growth factor (resulting in no, or at least reduced, scarring), as defined in the amended claims.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The description is not in strict agreement with the amended claims.

R.D

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
M92/0120/PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB93/00586	22/03/93	28/03/92
Applicant		
THE VICTORIA UNIVERSITY OF MANCHESTER et al.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [X] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:
   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 93/00586

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K37/02; A61K39/395; C07K15/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K ; C07K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

Shah et al Lancet 339, 213-214 '92

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X,P	WO,A,9 217 206 (THE VICTORIA UNIVERSITY OF MANCHESTER) 15 October 1992 see page 4, line 11 - page 13, line 23	1,3,4-19
X	WO,A,9 003 810 (ED GEISTLICH SÖHNE AG FÜR CHEMISCHE INDUSTRIE) 19 April 1990 see page 1, line 1 - line 19	1,3
X	EP,A,0 375 127 (GENENTECH) 27 June 1990 see column 5, line 41 - line 51 see column 7, line 21 - column 12, line 16	1,2,6-19

-/--

45

1-7, 12-14, 20

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
14 JULY 1993	30. 07. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	REMPP G.L.E.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB93/00586

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 19 is directed to a method of treatment of the humsn/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

GB 9300586
SA 72604

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 14/07/93

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9217206	15-10-92	AU-A-	1436892	02-11-92
WO-A-9003810	19-04-90	None		
EP-A-0375127	27-06-90	AU-A-	4524889	28-05-90
		CA-A-	2002130	02-05-90
		WO-A-	9004974	17-05-90
WO-A-9110727	25-07-91	None		
EP-A-0433225	19-06-91	AU-A-	6701890	13-06-91
		JP-A-	3191791	21-08-91

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

GB 9300586
SA 72604

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 14/07/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9217206	15-10-92	AU-A- 1436892	02-11-92
WO-A-9003810	19-04-90	None	
EP-A-0375127	27-06-90	AU-A- 4524889	28-05-90
		CA-A- 2002130	02-05-90
		WO-A- 9004974	17-05-90
WO-A-9110727	25-07-91	None	
EP-A-0433225	19-06-91	AU-A- 6701890	13-06-91
		JP-A- 3191791	21-08-91

INTERNATIONAL SEARCH REPORT

rnational application No.
PCT/GB93/00586

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 19 is directed to a method of treatment of the humsn/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No PCT/NO 90/00173

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: E 01 D 11/00, 21/04

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	E 01 D

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	FR, A1, 2580687 (WIECZOREK, J.) 24 October 1986, see page 15, line 16 - line 40; figures 28-31	1-7, 10
A	--	8,9, 11
A	DE, A1, 1658631 (FA. STRABAG BAU-AG) 29 October 1970, see page 7 last paragraph - page 8 first paragraph; figures 3-5	1-11
	--	
A	US, A, 3832748 (OGLETREE, W.B.) 3 September 1974, see column 2, line 63 - column 3, line 17; figure 1	1-11
	--	
	--------	

* Special categories of cited documents:[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
18th February 1991	1991 -02- 26
International Searching Authority	**Signature of Authorized Officer**
SWEDISH PATENT OFFICE	Ingemar Hedlund

Form PCT/ISA/210 (second sheet) (January 1985)

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/NO 90/00173

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the Swedish Patent Office EDP file on 91-01-31
The Swedish Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR-A1- 2580687	86-10-24	FR-A- 2589178	87-04-30
DE-A1- 1658631	70-10-29	NONE	
US-A- 3832748	74-09-03	NONE	

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 93/00586

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K37/02; A61K39/395; C07K15/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K ; C07K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X,P	WO,A,9 217 206 (THE VICTORIA UNIVERSITY OF MANCHESTER) 15 October 1992 see page 4, line 11 - page 13, line 23 ---	1,3,4-19
X	WO,A,9 003 810 (ED GEISTLICH SÖHNE AG FÜR CHEMISCHE INDUSTRIE) 19 April 1990 see page 1, line 1 - line 19 ---	1,3
X	EP,A,0 375 127 (GENENTECH) 27 June 1990 see column 5, line 41 - line 51 see column 7, line 21 - column 12, line 16 ---	1,2,6-19

-/--

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
14 JULY 1993	29. 07. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	REMPP G.L.E.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	WO,A,9 110 727 (LA JOLLA CANCER RESEARCH FOUNDATION) 25 July 1991 see page 10, line 25 - page 11, line 11 see page 22, line 6 - page 23, line 21	1,4,5
X	EP,A,0 433 225 (CIBA-GEIGY AG) 19 June 1991 cited in the application see page 4, line 29 - page 5, line 19 see page 8, line 22 - page 9, line 39	1,2,6, 13-19

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	WO,A,9 110 727 (LA JOLLA CANCER RESEARCH FOUNDATION) 25 July 1991 see page 10, line 25 - page 11, line 11 see page 22, line 6 - page 23, line 21	1,4,5
X	EP,A,0 433 225 (CIBA-GEIGY AG) 19 June 1991 cited in the application see page 4, line 29 - page 5, line 19 see page 8, line 22 - page 9, line 39	1,2,6, 13-19

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING
SUBMISSION OF PRIORITY DOCUMENTS

(PCT Administrative Instructions, Section 411)

To:

McNEIGHT, David, Leslie
McNeight & Lawrence
Regent House
Heaton Lane
Stockport, Cheshire SK4 1BS
ROYAUME-UNI

Date of mailing:
27 May 1993 (27.05.93)

Applicant's or agent's file reference:
M92/0120/PCT

IMPORTANT NOTIFICATION

International application No.:	International filing date:	Priority date:
PCT/GB93/00586	22 March 1993 (22.03.93)	28 March 1992 (28.03.92)

Applicant:
THE VICTORIA UNIVERSITY OF MANCHESTER et al

The applicant is hereby notified of the date of receipt by the International Bureau of the priority document(s) relating to the following application(s):

Priority application No:	Priority date:	Priority country:	Date of receipt of priority document:
9206861.8	28 Mar 1992 (28.03.92)	GB	27 May 1993 (27.05.93)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorised officer:

B. Fitzgerald

Telephone No.: (41-22) 730.91.11

PATENT COOPERATION TREATY

PCT

08/307640

55 Rec'd PCT/PTO 16 SEP 1994

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference **M92/0120/PCT**	**For Further Action**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International Application No. **PCT/GB 93/00586**	International Filing Date *(day/month/year)* **22 March 1993**	Priority Date *(day/month/year)* **28 March 1992**
International Patent Classification (IPC) **IPC5: A61K 37/02, 39/395; C07K 15/00**		
Applicant **THE VICTORIA UNIVERSITY OF MANCHESTER et al**		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This **REPORT** consists of a total of **5** sheets.

☒ This report is also accompanied by **ANNEXES** i.e., sheets of the description, claims and/or drawings amended during international preliminary examination and/or containing rectifications made before this Authority.

These annexes consist of a total of **4** sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

Date of submission of the demand **22 March 1993**	Date of completion of this report **24 February 1993**
Name and mailing address of the IPEA **The Patent Office, Cardiff Road, NEWPORT, Gwent, NP9 1RH**	Authorized Officer **C Sherrington**
Facsimile No **0633 814444**	Telephone No **0633 814965**

Form PCT/IPEA/409 (first sheet) (July 1992)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International Application No
PCT/GB 93/00586

I. Basis of the report

1. This report has been drawn on the basis of:

☐ the international application as originally filed.

☒ the description, pages 1-17, as originally filed,
pages, filed with the demand,
pages, filed with the letter of
pages, filed with the letter of

☒ the claims, pages, as originally filed,
pages, as amended under Article 19,
pages, filed with the demand,
pages 18-21, filed with the letter of 21 February 1994
pages, filed with the letter of

☐ the drawings, sheets, as originally filed,
sheets, filed with the demand,
sheets, filed with the letter of
sheets, filed with the letter of

2. The amendments have resulted in the cancellation of: pages:
sheets of drawings No:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box.

4. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

☐ copy of the earlier application whose priority has been claimed.

☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application,

☒ claims Nos. 20

because:

☒ the said international application, or the said claims Nos. 20 relate to the following subject matter which does not require an international preliminary examination *(specify)*:

Method of treatment by therapy of the human or animal body - PCT rule 67.1(iv).

☐ the description, claims or drawings (indicate particular elements below) or said claims Nos . are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims or said claims Nos . are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for said claims Nos .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	claims 1 to 19	YES
	claims	NO
Inventive Step (IS)	claims 1 to 19	YES
	claims	NO
Industrial Applicability (IA)	claims 1 to 19	YES
	claims	NO

2. CITATIONS AND EXPLANATIONS

None of the cited documents either specifically disclose, or, taken together or separately, are considered to lead to, the wound healing compositions, which comprise at least one non-fibrotic growth factor (resulting in no, or at least reduced, scarring), as defined in the amended claims.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The description is not in strict agreement with the amended claims.

RECORD COPY. NO FEES.

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No. PCT/GB 93 / 00586

International Filing Date 22 - 03 - 93 (22 MARCH 1993)

United Kingdom Patent Office PCT International Application

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference (if desired) (12 characters maximum) M92/0120/PCT

Box No. I TITLE OF INVENTION

Wound Healing and Treatment of Fibrotic Disorders

Box No. II APPLICANT

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

The Victoria University of Manchester
Oxford Road
Manchester M13 9PT
~~Great Britain~~ AA/A GB

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State (i.e. country) of nationality: ~~Great Britain~~ AA/A GB

State (i.e. country) of residence: ~~Great Britain~~ AA/A GB

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

AA/A AMENDED RO/GB.

FERGUSON, Mark William James
13 Peel Moat Road
Heaton Moor
Stockport
Cheshire SK4 4PL
~~Great Britain~~ AA/A GB

This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below.)

State (i.e. country) of nationality: ~~Great Britain~~ AA/A GB

State (i.e. country) of residence: ~~Great Britain~~ AA/A GB.

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

SHAH, Mamta
12 Lathom Road
Withington
Manchester M20 9NX
~~Great Britain~~ AA/A GB.

This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below.)

State (i.e. country) of nationality: India

State (i.e. country) of residence: ~~Great Britain~~ AA/A GB

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: [X] agent [ ] common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

McNEIGHT, David Leslie
LAWRENCE, John Gordon
McNeight & Lawrence
Regent House, Heaton Lane
Stockport, Cheshire, SK4 1BS
~~Great Britain~~ GB.

AA/A
AMENDED
RO/GB.

Telephone No.: 061 480 6394

Facsimile No.: 061 480 2622

Teleprinter No.: 669226 DENPAT G

[ ] Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

[X] **EP** **European Patent:** **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **DE** Germany, **DK** Denmark, **ES** Spain, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

[X] **OA** **OAPI Patent:** Benin, Burkina Faso, Cameroon, Central African Republic, Chad, Congo, Côte d'Ivoire, Gabon, Guinea, Mali, Mauritania, Senegal, Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

[X] **AT** Austria .....
[X] **AU** Australia .....
[X] **BB** Barbados
[X] **BG** Bulgaria .....
[X] **BR** Brazil .....
[X] **CA** Canada
[X] **CH and LI** Switzerland and Liechtenstein
[X] **CZ** Czech Republic .....
[X] **DE** Germany .....
[X] **DK** Denmark .....
[X] **ES** Spain .....
[X] **FI** Finland
[X] **GB** United Kingdom
[X] **HU** Hungary .....
[X] **JP** Japan .....
[X] **KP** Democratic People's Republic of Korea
.....
[X] **KR** Republic of Korea .....
[X] **LK** Sri Lanka
[X] **LU** Luxembourg .....
[X] **MG** Madagascar
[X] **MN** Mongolia .....
[X] **MW** Malawi .....
[X] **NL** Netherlands
[X] **NO** Norway
[X] **NZ** New Zealand
[X] **PL** Poland .....
[X] **PT** Portugal .....
[X] **RO** Romania
[X] **RU** Russian Federation .....
[X] **SD** Sudan
[X] **SE** Sweden
[X] **SK** Slovak Republic .....
[X] **UA** Ukraine
[X] **US** United States of America .....
.....

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

[ ] .....
[ ] .....

In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designation(s) of _____.

The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

PCT/GB 93 / 00586

Box No. VI PRIORITY CLAIM Further priority claims are indicated in the Supplemental Box ☐

The priority of the following earlier application(s) is hereby claimed:

	Country (in which, or for which, the application was filed)	Filing Date (day/month/year)	Application No.	Office of filing (only for regional or international application)
item (1)	~~Great Britain~~ GB	28 — 03 — 92 28 March 1992	9206861.8	
item (2)				
item (3)				

AA|A. AMENDED RO|GB

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):

☒ The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s) : (1)

Box No. VII EARLIER SEARCH

Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request:

Country (or regional Office): Date *(day/month/year)*: Number: (Ref: RS 90820 GB)

European Patent Office 28.01.93 GB 9206861

Box No. VIII CHECK LIST

This international application contains the following number of sheets:

1. request	3	sheets
2. description	17	sheets
3. claims	4	sheets
4. abstract	1	sheets
5. drawings	–	sheets
Total	25	sheets

This international application is accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☐ priority document(s) *identified in Box No. VI as item(s):*
5. ☐ fee calculation sheet
6. ☐ separate indications concerning deposited microorganisms
7. ☐ nucleotide and/or amino acid sequence listing (diskette)
8. ☒ other *(specify)*: 24\77

Figure No. _____ of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

McNEIGHT, David Leslie - Agent

For receiving Office use only

1. Date of actual receipt of the purported international application: 22 MARCH 1993 | 22-03-93
2. Drawings: ☐ received: ☒ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority specified by the applicant: ISA/
6. ☒ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau: 06 APRIL 1993 (06.04.93)

WOUND HEALING AND TREATMENT OF FIBROTIC DISORDERS

This invention concerns the healing of wounds and other conditions in which fibrosis is a major mechanism of tissue repair or where excessive fibrosis leads to pathological derangement and malfunctioning of the tissues. It refers in particular to agents and techniques for facilitating repair and healing of animal tissues, without excessive fibrosis, and for preventing or treating diseases and conditions of fibrosis.

Fibrosis is a major problem in wound healing causing scarring of the tissue, which not only looks unsightly, but also causes problems in respect of growth of the tissue, function, movement etc. This is particularly true following injuries to children or following major burns.

In addition, fibrosis is a major medical problem where abnormal or excessive deposition of fibrous tissue occurs in many diseases, including liver cirrhosis, glomerulonephritis, pulmonary fibrosis, systemic fibrosis, rheumatoid arthritis, as well as wound healing.

The mechanism of fibrosis is still not fully understood, but wound healing usually begins as an

inflammatory reaction with leucocyte infiltration and accumulation of cytokines. These cytokines are responsible for the proliferation of fibroblasts and the deposition of extracellular matrix proteins (including collagen and fibronectin) which accumulate and result in permanent alteration in tissue structure and function.

Examples of the regulatory cytokines include tumor necrosis factor (TNF), fibroblast growth factors (FGF's), platelet derived growth factor (PDGF) and transforming growth factor ß (TGFß), (TGFß-1 to TGFß-5 have so far been identifeid). Two of these cytokines families, TGFß and PDGF, have been reported to be highly fibrogenic, and, moreover, inhibition of two of the TGFß's and PDGF activity, using anti-TGFß-1, anti-TGFß-2 and anti-PDGF antibodies, has been shown to diminish fibrosis in tissue injury (Shah el al, The Lancet, 339, 213-214, 1992; WO 91/04748).

The present invention provides novel compositions useful in the treatment of wounds and fibrotic disorders and which may prevent, inhibit or reverse fibrosis.

The invention comprises a healing composition containing at least one non-fibrotic growth factor in combination with a pharmaceutically acceptable carrier.

The composition may comprise TGFß-3 as the or a non-fibrotic growth factor, such that on application of the composition to the tissue, this non-fibrotic growth factor is present in an elevated level compared to its naturally occuring level.

The composition may comprise acidic or basic FGF as the or a non-fibrotic growth factor, again resulting in a much elevated level of non-fibrotic growth factor than would naturally be present.

The composition may comprise anti-fibrotic agents, such as fibrotic growth factor neutralising agents, for example antibodies to TGFß-1, TGFß-2 and PDGF; binding proteins which prevent TGFß-1, TGFß-2 and PDGF from binding to their receptors by either binding to the growth factor itself, eg. Decorin, Biglycan, or binding to the receptor, eg. peptides containing the receptor binding site sequence or soluble forms of the growth factor receptors and the growth factor binding domains of these receptors; and antisense oligo-nucleotides or ribosymes which act to prevent fibrotic growth factor mRNA translation.

The composition may comprise combinations of non-fibrotic growth factors, for example, TGFß-3 and anti-fibrotic agents, for example, anti-TGFß-1 and anti-TGFß-2.

The non-fibrotic growth factor and/or anti-fibrotic agent(s) may be present in the composition in an active or inactive form. Inactivation may be by

any of a number of mechanisms, for example, by encapsulation. Capsules may be degradeable by an external stimulus to release the active form when required. The external stimulus may include UV light, ultrasound, in vivo enzymes or heat.

Inactivation may, however, be by the molecular addition of a binding molecule. The binding molecule may be detachable when required by an external stimulus such as UV light, ultrasound, in vivo enzymes or heat.

The non-fibrotic growth factor may be present in an inactive form, for example, as a precursor, and may be activated upon contact with tissue containing the natural cleavage enzymes required to convert the precursor into its active form.

The carrier may comprise a neutral sterile cream, gel, aerosol or powder for topical application, or may be in the form of a patch, sterile dressing or an absorbable dressing. The carrier may be a biopolymer of collagen, hyaluronic acid or polymer of PVC to which the anti-fibrotic or non fibrotic agents are attached in such a way as to facilitate their action and/or release when the carrier is in contact with or implanted into either the wound or fibrotic lesion. The carrier may also comprise a sterile solution for irrigation,

injection either locally or systemically or inhalation, or may be in the form of a tablet, capsule, and the like, for enteral administration.

The present invention also provides a method of preparation of a pharmaceutical healing or anti-fibrotic composition containing at least one non-fibrotic growth factor for topical application in a cream, gel, aerosol, powder, patch, dressing, biopolymer or polymer implant, delay or slow release system, or in a solution for irrigation, injection or inhalation, or in a tablet or capsule for enteral administration.

The present invention also provides a method of inhibiting fibrosis during the healing of wounds and in other fibrotic conditions and disorders, for example ulcers, comprising administering to a host suffering from tissue wounding or other fibrotic conditions and disorders, at least one non-fibrotic growth factor.

The present invention also provides a method of reversing fibrosis in such fibrotic conditions and disorders comprising administering to a host suffering from such fibrotic conditions and disorders, at least one non-fibrotic growth factor, for example, TGFß-3 and/or at least one anti-fibrotic agent for example, anti-TGFß-1/TGFß-2.

As mentioned above, two cytokines have been identified as being involved in fibrosis, namely PDGF and TGFß. Of these two, TGFß appears to play the major role. For example, in tissues which heal without scar formation, such as fetal and embryonic wounds where there is a lowered inflammatory response and altered cytokine profile, the level of TGFß in particular, is much reduced.

TGFß comprises a family of molecules, the important mammalian members being TGFß-1, TGFß-2 and TGFß-3 (Roberts and Sporn, The Transforming Growth Factor-ßs, In: Peptide Growth Factors and their Receptors, Springer Verlag, Berlin, 1990, p418-472). The TGFßs, although having different patterns of expression, share over 70% peptide homology and are thought to have similar functions and act inter-changeably. Thus in wound healing it would be expected that TGFß-3 would act like TGFß-1 and TGFß-2 to increase extracellular matrix production, angiogenesis and the inflammatory response.

As discussed above, fibrotic disease is a major medical problem. In such diseases, there is abnormal or excessive deposition of fibrous tissue. Such diseases are exemplified by liver cirrhosis, glomerulonephritis, pulmonary fibrosis, systemic fibrosis and rheumatoid

arthritis. In such diseases the use of TGFß would be avoided, since TGFß's are believed to increase the deposition of fibrous tissue. Suprisingly, it has now been discovered that TGFß-3 has the opposite effect to that expected, in that it promotes healing without promoting the deposition of fibrous tissue.

The present invention provides the use of a TGFß-3 for the manufacture of a medicament for the treatment of a fibrotic disease.

The present invention also provides a method of treating a fibrotic disease by administering a pharmaceutically effective amount of TGFß-3 to a patient in need thereof.

The present invention also provides an agent for treating a fibrotic disease which comprises TGFß-3 as active ingredient.

The present invention also provides a pharmaceutical composition comprising a higher proportion of TGFß-3 in relation to TGFß-1 or TGFß-2, compared with relative proportions in naturally occuring TGFß, and a pharmaceutically acceptable carrier.

EP 0 433 225 defines the biological activity of the TGFß's ie. TGFß-1, TGFß-2 and TGFß-3, as including the ability to increase formation of fibrous granular tissue in and around wound implants in rats (page 5, lines 17-19), while US 4,810,691 and US 4,77,228 describe the use of TGFß's for promoting connective tissue deposition.

Experiments described in detail below indicate that contrary to the conventional view that TGFß-3 acts in the same manner as TGFß-1 and TGFß-2 to increase fibrosis at the site of wound healing, it has in fact the opposite effect and promotes wound healing with reduced fibrosis and scarring.

Experiments

The experiments have involved exogenous injection of TGFß-1, TGFß-2 or TGFß-3. They have also involved the injection of neutralising antibodies to TGFß-1 or TGFß-2 (or anti TGFß-1 and TGFß-2 in combination) . Neutralising antibodies to TGFß-3 are not yet available. The experimental protocol was as described in Shah et al, The Lancet, 339, 213-214, 1992)

These experiments produced a very interesting and unexpected set of results. First, the neutralising

antibody to TGFß-1 diminished scarring, i.e. reduced the amount of extracellular matrix, reduced angiogenesis and reduced the numbers of macrophages and monocytes at the wound. It also improved the orientation of collagen fibres in the healing wound. The neutralising antibody to TGFß-2 had very little effect on its own, but showed a slight improvement in scarring. Combined, the neutralising antibodies to TGFß-1 and TGFß-2 showed a marked improvement in wound healing (similar to that described in Shah et al, The Lancet, 339, 213-214, 1992), namely decreased extracellular matrix deposition (decreased fibronectin, decreased collagen), decreased angiogenesis, decreased macrophages and monocytes at the wound site and better orientation of collagen and fibronectin within the wound. Exogenous addition of TGFß-1 or TGFß-2 had the expected result, namely of increasing extracellular matrix deposition, increasing angiogenesis and increasing the inflammatory response. However, exogenous addition of TGFß-3 did not have this effect, but rather produced effects similar to those observed with the neutralising antibodies to TGFß-1 and TGFß-2, namely, a reduction in the amount of extracellular matrix deposited, a decrease in macrophages and monocytes and a marked improvement in subsequent scarring.

Specific details of the experiments to document the TGFß-3 effect are as follows :-

Adult male Sprague-Dawley rats (200 to 250 gram weight) were anaesthetised with halothane nitrous oxide and oxygen inhalation. Two incisions, ten millimetres in length and to the depth of the parniculous carnosis were made in the dorsal skin, equal distant from the midline and between the fore and hind limbs. The wounds were left unsutured to heal by secondary intention to produce the greatest amount of granulation tissue and scarring. In each animal, one wound (control) was unmanipulated. In different animals the other wound received a) an injection of transforming growth factor beta 1 (TGFß-1) (20 ng per injection), or b) an injection of TGFß-2 (20 ng per injection) or c) an injection of TGFß-3 (20 ng per injection). It had previously been determined from dose response experiments that 20 ng per injection was the optimum dose to give. Injections were of 100 microlitres in phosphate buffered saline and were introduced into the wound margins by local infiltration on days 0, 1 and 2. The fluid was infiltrated along the length of each wound margin through a single entry point 0.5 cm distal to the caudal end of the wound. At least four animals were killed by chloroform overdose on each of days 7, 14 and 42 after wounding. The wounds were processed for

routine histological examination, particularly using connective tissue stains such as Mallory or Masson's trichrome. They were also processed for immuno-cytochemistry, using antibodies to detect fibronectin (as a marker of early wound repair and to show the orientation of extracellular matrix molecules), macrophages and monocytes (as an indication of the inflammatory response), laminim (to highlight basement membranes, e.g. of newly formed blood vessels) and collagen types I and III to document connective tissue deposition within the wound and scarring.

Summary of Results

Compared to control wounds, at 7 and 14 days, the TGFß-3 treated wounds had less fibronectin and the fibronectin fibres were in a beter orientation. By six weeks, the fibronectin in all wounds was similar in quantity to that of the surrounding normal skin. However, that in the TGFß-3 treated wound had a much better orientation than the other wounds. The results were almost indistinguishable from the results obtained with neutralising antibodies to TGFß-1 and TGFß-2. By comparison, wounds treated with TGFß-1 or TGFß-2 showed a vastly increased quantity of fibronectin in the wound at 7 days and this fibro- nectin had an abnormal orientation, compared to the surrounding tissue. The

same was true at 14 days, but by 6 weeks there was little difference between the TGFß-1 or TGFß-2 treated wounds and the control in terms of the quantity of fibronectin present.

At 7 days TGFß-1 treated and TGFß-2 treated and control wounds showed similar profile of macrophage and monocyte infiltration (for example control 159, TGFß-1 149, control 117, TGFß-2 112 per section). However, TGFß-3 treated wounds had a low profile of macrophage plus monocyte infiltration (control 130, TGFß-3 91 per section).

At 7 days TGFß-1 treated and TGFß-2 treated wounds had a higher proliferation of macrophages in the lower half of the wounds compared to similar areas in the control wounds (control 50/TGFß-1 80, control 45/ TGFß-2 59 per section). However, in the upper half of the wounds the macrophage infiltration was similar in the TGFß-1 treated and control wounds (control 37, TGFß-1 39 per section) whilst TGFß-2 treated wounds had a lower profile (control 34, TGFß-2 19). By contrast, TGFß-3 treated wounds showed a lower macrophage profile throughout the entire wound, compared to the control wounds (upper half control 41, TGFß-3 16; lower half control 72, TGFß-3 28 per section).

Laminin staining was used as a marker of neovascularisation. At 7 days, TGFß-1 treated wounds showed an increase in the number of blood vessels, particularly at the base of the wound. TGFß-2 treated wounds appeared similar to the control wounds. TGFß-3 treated wounds, however, had many more blood vessels compared to either the control or the TGFß-1 or the TGFß-2 treated wounds. This was a very marked effect.

By 14 days there were few differences in the number of blood vessels between either the TGFß-1, TGFß-2 or TGFß-3 treated wounds compared to the control. However, the TGFß-3 treated wounds tended to have more blood vessels.

In terms of collagen deposition within the wound, as assayed by Mallory staining or immunocytochemistry, treatment of the wound with either TGFß-1 or TGFß-2 increased the amount of collagen within the wound on days 7 and 14 after wounding. Furthermore, this collagen had an abnormal orientation with a much higher percentage of fibres orientated in a vertical direction, compared to the surrounding dermis. At six weeks, the control, TGFß-1 and TGFß-2 treated wounds were visibly scarred with an abnormal accumulation of abnormally orientated collagen within the wounded area. By contrast, wounds treated with TGFß-3 showed slightly

less collagen deposition on days 7 and 14 after wounding. Moreover, the collagen deposited was in a similar reticular pattern to that of the surrounding dermis. Consequently, by six weeks after wounding, the TGFß-3 treated wounds had a more similar dermal architecture to that of the surrounding normal skin, compared to either the control TGFß-1 or TGFß-2 treated wounds. This result with TGFß-3 is very similar to that obtained with neutralising antibodies to TGFß-1 and TGFß-2.

In summary, therefore, treatment of the wounds with TGFß-3 decreased the amount of extracellular matrix deposited in the early wound, assured that the orientation of this matrix was in the normal reticular pattern of the dermis, compared to the abnormal pattern of the scar, decreased the number of macrophages and monocytes and hence inflammatory infiltrate into the wound, but greatly increased the number of blood vessels in the early healing wound. These effects are almost identical to those observed with neutralising antibodies to TGFß-1 and TGFß-2 except the increase in the number of blood vessels. Treatment of the wounds with neutralising antibodies to TGFß-1 and TGFß-2 decrease the amount of extracellular matrix deposited, alter the orientation of this matrix, so that it is in a more normal alignment, decrease the inflammatory infiltrate

of macrophages and monocytes (like TGFß-3) but decrease the number of blood vessels (unlike treatment with TGFß-3 which increases the number of blood vessels).

TGFß-3 therefore acts as an anti-scarring (anti-fibrotic) agent. It is very clear that this is an isoform specific effect within the TGFß family.

TGFß-3 therefore becomes a target as an anti-fibrotic agent or an anti-scarring agent. It may be capable of biological modification to increase the anti-fibrotic effect or define more carefully that portion of the molecule responsible for these effects. It may be possible to optimise the structure of TGFß-3 as an anti-fibrotic agent, based on such analysis. The effects of TGFß-3 in this regard are unpredictable from the literature, and interestingly, differ from the neutralising antibody experiments, particularly in the increase in angiogenesis. This may actually be beneficial for certain kinds of wound healing, e.g. chronic wounds such as venous leg ulcers, where one wants to increase the vascular supply to stimulate healing but decrease subsequent scarring.

In the context of fibrosis, the efffects of TGFß-3 or anti TGFß-1/TGFß-2 agents are not limited to preventing further increases of fibrosis. TGFß-1/TGFß-2

act to increase the accumulation of extracellular matrix molecules both by stimulating synthesis of new extracellular matrix molecules and decreasing the removal of existing matrix molecules, i.e. inhibiting tissue turnover (Roberts and Sporn, the transforming growth factor - ß's, In: Peptide growth factors and their receptors, Springer Verlag, Berlin, 1990, p 418-472). Therefore, any agent which antagonises or neutralises or renders ineffective TGFß-1/TGFß-2 not only decreases extracellular matrix synthesis but also increases remodelling. As an anti-fibrotic agent either TGFß-3 or anti-TGFß-1/anti- TGFß-2/anti-PDGF (or some combination thereof) may in certain fibrotic diseases, e.g. glomerulonephritis, pulmonary fibrosis, reverse the accumulation of fibrous scar tissue already present in the tissue.

It will be appreciated that it is not intended to limit the invention to the above examples only, many variations, such as might readily occur to one skilled in the art, being possible, without departing from the scope thereof as defined in the appended claims.

Thus for example, as well as applying a preparation to a wound containing TGFß-3 only, this may be given in combination with fibrotic growth factor neutralising agent(s), for example, anti-TGFß-1 and/or

anti-TGFß-2 and/or anti-PDGF antibodies, in a ratio which will enable the required amount of vascularisation for the particular type of wound to be provided whilst at the same time healing the wound without scarring.

CLAIMS

1. A healing composition containing at least one non-fibrotic growth factor in combination with a pharmaceutically acceptable carrier.

2. A composition according to claim 1, wherein the non-fibrotic growth factor comprises TGFß-3.

3. A composition according to claim 1, wherein the non-fibrotic growth factor comprises FGF.

4. A composition according to any preceding claim, comprising anti-fibrotic agents.

5. A composition according to claim 4, wherein the anti-fibrotic agents include antibodies to TGFß-1, TGFß-2 and PDGF; binding proteins which prevent TGFß-1, TGFß-2 and PDGF from binding to their receptors by either binding to the growth factor itself, eg. Decorin, Biglycan, or binding to the receptor, eg. peptides containing the receptor binding site sequence; or soluble forms of growth factor receptor or the growth factor binding domains of these receptors or antisense oligonucleotides or ribosymes which act to prevent fibrotic growth factor mRNA translation.

6. A composition according to any preceding claim wherein the non-fibrotic growth factor and/or anti-fibrotic agent(s) are present in the composition in an active form.

7. A composition according to any of claims 1 to 5, wherein the non-fibrotic growth factor and/or anti-fibrotic agent(s) are present in the composition in an inactive form.

8. A composition according to claim 7, wherein inactivation is by encapsulation.

9. A composition according to claim 8, wherein the capsules are degradeable by an external stimulus to release the active form when required.

10. A composition according to claim 9, wherein the external stimulus includes UV light, ultrasound, *in vivo* enzymes or heat.

11. A composition according to claim 7, wherein inactivation is by the molecular addition of a binding molecule which is detachable when required by an external stimulus including UV light, ultrasound, *in vivo* enzymes or heat.

12. A composition according to any preceding claim, wherein the non-fibrotic growth factor is present in an inactive form, for example, as a precursor, and is activated upon contact with tissue containing the natural cleavage enzymes required to convert the precursor into its active form.

13. A composition according to claim 1, wherein the carrier comprises a neutral sterile cream, gel, aerosol or powder for topical application.

14. A composition according to claim 1, wherein the carrier comprises a patch or a sterile dressing or an absorbable dressing for topically covering a wound.

15. A composition according to claim 1, wherein the carrier comprises a sterile solution for irrigation, injection or inhalation.

16. A composition according to claim 1, wherein the carrier comprises a tablet, capsule, and the like, for enteral administration.

17. A composition according to claim 1, wherein the carrier comprises a biopolymer, for example collagen, hyaluronic acid or polymer, for contacting or implanting into the wound/fibrotic lesion so as to allow release of

the active agents slowly or quickly and for to be active in situ.

18. A method of preparation of a pharmaceutical healing or anti-fibrotic composition containing at least one non-fibrotic growth factor for topical application in a cream, gel, powder, aerosol, patch or dressing, biopolymer or polymer implant, delay or slow release system or in a solution for irrigation, injection or inhalation, or in a tablet or capsule for enteral administration.

19. A method of inhibiting fibrosis during the healing of wounds and other fibrotic diseases, disorders or conditions, comprising administering to a host suffering from tissue wounding or these fibrotic conditions, at least one non-fibrotic growth factor.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
A61K 37/02, 39/395, C07K 15/00

A1

(11) International Publication Number: **WO 93/19769**

(43) International Publication Date: 14 October 1993 (14.10.93)

(21) International Application Number: PCT/GB93/00586

(22) International Filing Date: 22 March 1993 (22.03.93)

(30) Priority data:
9206861.8 28 March 1992 (28.03.92) GB

(71) Applicant *(for all designated States except US):* THE VICTORIA UNIVERSITY OF MANCHESTER [GB/GB]; Oxford Road, Manchester M13 9PT (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only) :* FERGUSON, Mark, William, James [GB/GB]; 13 Peel Moat Road, Heaton Moor, Stockport, Cheshire SK4 4PL (GB). SHAH, Mamta [IN/GB]; 12 Lathom Road, Withington, Manchester M20 9NX (GB).

(74) Agents: McNEIGHT, David, Leslie et al.; McNeight & Lawrence, Regent House, Heaton Lane, Stockport, Cheshire SK4 1BS (GB).

(81) Designated States: AT, AU, BB, BG, BR, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: WOUND HEALING AND TREATMENT OF FIBROTIC DISORDERS

(57) Abstract

A healing composition containing at least one non-fibrotic growth factor in combination with a pharmaceutically acceptable carrier is disclosed. A method of preparation of the composition and method of treating a host, suffering from a wound or fibrotic condition, disease, disorder with the composition is also disclosed.

WOUND HEALING AND TREATMENT OF FIBROTIC DISORDERS

This invention concerns the healing of wounds and other conditions in which fibrosis is a major mechanism of tissue repair or where excessive fibrosis leads to pathological derangement and malfunctioning of the tissues. It refers in particular to agents and techniques for facilitating repair and healing of animal tissues, without excessive fibrosis, and for preventing or treating diseases and conditions of fibrosis.

Fibrosis is a major problem in wound healing causing scarring of the tissue, which not only looks unsightly, but also causes problems in respect of growth of the tissue, function, movement etc. This is particularly true following injuries to children or following major burns.

In addition, fibrosis is a major medical problem where abnormal or excessive deposition of fibrous tissue occurs in many diseases, including liver cirrhosis, glomerulonephritis, pulmonary fibrosis, systemic fibrosis, rheumatoid arthritis, as well as wound healing.

The mechanism of fibrosis is still not fully understood, but wound healing usually begins as an

inflammatory reaction with leucocyte infiltration and accumulation of cytokines. These cytokines are responsible for the proliferation of fibroblasts and the deposition of extracellular matrix proteins (including collagen and fibronectin) which accumulate and result in permanent alteration in tissue structure and function.

Examples of the regulatory cytokines include tumor necrosis factor (TNF), fibroblast growth factors (FGF's), platelet derived growth factor (PDGF) and transforming growth factor ß (TGFß), (TGFß-1 to TGFß-5 have so far been identifeid). Two of these cytokine families, TGFß and PDGF, have been reported to be highly fibrogenic, and, moreover, inhibition of two of the TGFß's and PDGF activity, using anti-TGFß-1, anti-TGFß-2 and anti-PDGF antibodies, has been shown to diminish fibrosis in tissue injury (Shah el al, The Lancet, 339, 213-214, 1992; WO 91/04748).

The present invention provides novel compositions useful in the treatment of wounds and fibrotic disorders and which may prevent, inhibit or reverse fibrosis.

The invention comprises a healing composition containing at least one non-fibrotic growth factor in combination with a pharmaceutically acceptable carrier.

The composition may comprise TGFß-3 as the or a non-fibrotic growth factor, such that on application of the composition to the tissue, this non-fibrotic growth factor is present in an elevated level compared to its naturally occuring level.

The composition may comprise acidic or basic FGF as the or a non-fibrotic growth factor, again resulting in a much elevated level of non-fibrotic growth factor than would naturally be present.

The composition may comprise anti-fibrotic agents, such as fibrotic growth factor neutralising agents, for example antibodies to TGFß-1, TGFß-2 and PDGF; binding proteins which prevent TGFß-1, TGFß-2 and PDGF from binding to their receptors by either binding to the growth factor itself, eg. Decorin, Biglycan, or binding to the receptor, eg. peptides containing the receptor binding site sequence or soluble forms of the growth factor receptors and the growth factor binding domains of these receptors; and antisense oligo-nucleotides or ribosymes which act to prevent fibrotic growth factor mRNA translation.

The composition may comprise combinations of non-fibrotic growth factors, for example, TGFß-3 and anti-fibrotic agents, for example, anti-TGFß-1 and anti-TGFß-2.

The non-fibrotic growth factor and/or anti-fibrotic agent(s) may be present in the composition in an active or inactive form. Inactivation may be by

any of a number of mechanisms, for example, by encapsulation. Capsules may be degradeable by an external stimulus to release the active form when required. The external stimulus may include UV light, ultrasound, in vivo enzymes or heat.

Inactivation may, however, be by the molecular addition of a binding molecule. The binding molecule may be detachable when required by an external stimulus such as UV light, ultrasound, in vivo enzymes or heat.

The non-fibrotic growth factor may be present in an inactive form, for example, as a precursor, and may be activated upon contact with tissue containing the natural cleavage enzymes required to convert the precursor into its active form.

The carrier may comprise a neutral sterile cream, gel, aerosol or powder for topical application, or may be in the form of a patch, sterile dressing or an absorbable dressing. The carrier may be a biopolymer of collagen, hyaluronic acid or polymer of PVC to which the anti-fibrotic or non fibrotic agents are attached in such a way as to facilitate their action and/or release when the carrier is in contact with or implanted into either the wound or fibrotic lesion. The carrier may also comprise a sterile solution for irrigation,

injection either locally or systemically or inhalation, or may be in the form of a tablet, capsule, and the like, for enteral administration.

The present invention also provides a method of preparation of a pharmaceutical healing or anti-fibrotic composition containing at least one non-fibrotic growth factor for topical application in a cream, gel, aerosol, powder, patch, dressing, biopolymer or polymer implant, delay or slow release system, or in a solution for irrigation, injection or inhalation, or in a tablet or capsule for enteral administration.

The present invention also provides a method of inhibiting fibrosis during the healing of wounds and in other fibrotic conditions and disorders, for example ulcers, comprising administering to a host suffering from tissue wounding or other fibrotic conditions and disorders, at least one non-fibrotic growth factor.

The present invention also provides a method of reversing fibrosis in such fibrotic conditions and disorders comprising administering to a host suffering from such fibrotic conditions and disorders, at least one non-fibrotic growth factor, for example, TGFß-3 and/or at least one anti-fibrotic agent for example, anti-TGFß-1/TGFß-2.

As mentioned above, two cytokines have been identified as being involved in fibrosis, namely PDGF and TGFß. Of these two, TGFß appears to play the major role. For example, in tissues which heal without scar formation, such as fetal and embryonic wounds where there is a lowered inflammatory response and altered cytokine profile, the level of TGFß in particular, is much reduced.

TGFß comprises a family of molecules, the important mammalian members being TGFß-1, TGFß-2 and TGFß-3 (Roberts and Sporn, The Transforming Growth Factor-ßs, In: Peptide Growth Factors and their Receptors, Springer Verlag, Berlin, 1990, p418-472). The TGFßs, although having different patterns of expression, share over 70% peptide homology and are thought to have similar functions and act inter-changeably. Thus in wound healing it would be expected that TGFß-3 would act like TGFß-1 and TGFß-2 to increase extracellular matrix production, angiogenesis and the inflammatory response.

As discussed above, fibrotic disease is a major medical problem. In such diseases, there is abnormal or excessive deposition of fibrous tissue. Such diseases are exemplified by liver cirrhosis, glomerulonephritis, pulmonary fibrosis, systemic fibrosis and rheumatoid

arthritis. In such diseases the use of TGFß would be avoided, since TGFß's are believed to increase the deposition of fibrous tissue. Suprisingly, it has now been discovered that TGFß-3 has the opposite effect to that expected, in that it promotes healing without promoting the deposition of fibrous tissue.

The present invention provides the use of a TGFß-3 for the manufacture of a medicament for the treatment of a fibrotic disease.

The present invention also provides a method of treating a fibrotic disease by administering a pharmaceutically effective amount of TGFß-3 to a patient in need thereof.

The present invention also provides an agent for treating a fibrotic disease which comprises TGFß-3 as active ingredient.

The present invention also provides a pharmaceutical composition comprising a higher proportion of TGFß-3 in relation to TGFß-1 or TGFß-2, compared with relative proportions in naturally occuring TGFß, and a pharmaceutically acceptable carrier.

EP 0 433 225 defines the biological activity of the TGFß's ie. TGFß-1, TGFß-2 and TGFß-3, as including the ability to increase formation of fibrous granular tissue in and around wound implants in rats (page 5, lines 17-19), while US 4,810,691 and US 4,774,228 describe the use of TGFß's for promoting connective tissue deposition.

Experiments described in detail below indicate that contrary to the conventional view that TGFß-3 acts in the same manner as TGFß-1 and TGFß-2 to increase fibrosis at the site of wound healing, it has in fact the opposite effect and promotes wound healing with reduced fibrosis and scarring.

Experiments

The experiments have involved exogenous injection of TGFß-1, TGFß-2 or TGFß-3. They have also involved the injection of neutralising antibodies to TGFß-1 or TGFß-2 (or anti TGFß-1 and TGFß-2 in combination) . Neutralising antibodies to TGFß-3 are not yet available. The experimental protocol was as described in Shah et al, The Lancet, 339, 213-214, 1992)

These experiments produced a very interesting and unexpected set of results. First, the neutralising

antibody to TGFß-1 diminished scarring, i.e. reduced the amount of extracellular matrix, reduced angiogenesis and reduced the numbers of macrophages and monocytes at the wound. It also improved the orientation of collagen fibres in the healing wound. The neutralising antibody to TGFß-2 had very little effect on its own, but showed a slight improvement in scarring. Combined, the neutralising antibodies to TGFß-1 and TGFß-2 showed a marked improvement in wound healing (similar to that described in Shah et al, The Lancet, 339, 213-214, 1992), namely decreased extracellular matrix deposition (decreased fibronectin, decreased collagen), decreased angiogenesis, decreased macrophages and monocytes at the wound site and better orientation of collagen and fibronectin within the wound. Exogenous addition of TGFß-1 or TGFß-2 had the expected result, namely of increasing extracellular matrix deposition and angiogenesis. However, exogenous addition of TGFß-3 did not have this effect, but rather produced effects similar to those observed with the neutralising antibodies to TGFß-1 and TGFß-2, namely, a reduction in the amount of extracellular matrix deposited, a decrease in macrophages and monocytes and a marked improvement in subsequent scarring.

Specific details of the experiments to document the TGFß-3 effect are as follows :-

Adult male Sprague-Dawley rats (200 to 250 gram weight) were anaesthetised with halothane nitrous oxide and oxygen inhalation. Two incisions, ten millimetres in length and to the depth of the parniculous carnosis were made in the dorsal skin, equal distant from the midline and between the fore and hind limbs. The wounds were left unsutured to heal by secondary intention to produce the greatest amount of granulation tissue and scarring. In each animal, one wound (control) was unmanipulated. In different animals the other wound received a) an injection of transforming growth factor beta 1 (TGFß-1) (20 ng per injection), or b) an injection of TGFß-2 (20 ng per injection) or c) an injection of TGFß-3 (20 ng per injection). It had previously been determined from dose response experiments that 20 ng per injection was the optimum dose to give. Injections were of 100 microlitres in phosphate buffered saline and were introduced into the wound margins by local infiltration on days 0, 1 and 2. The fluid was infiltrated along the length of each wound margin through a single entry point 0.5 cm distal to the caudal end of the wound. At least four animals were killed by chloroform overdose on each of days 7, 14 and 42 after wounding. The wounds were processed for

routine histological examination, particularly using connective tissue stains such as Mallory or Masson's trichrome. They were also processed for immuno-cytochemistry, using antibodies to detect fibronectin (as a marker of early wound repair and to show the orientation of extracellular matrix molecules), macrophages and monocytes (as an indication of the inflammatory response), laminin (to highlight basement membranes, e.g. of newly formed blood vessels) and collagen types I and III to document connective tissue deposition within the wound and scarring.

Summary of Results

Compared to control wounds, at 7 and 14 days, the TGFß-3 treated wounds had less fibronectin and the fibronectin fibres were in a beter orientation. By six weeks, the fibronectin in all wounds was similar in quantity to that of the surrounding normal skin. However, that in the TGFß-3 treated wound had a much better orientation than the other wounds. The results were almost indistinguishable from the results obtained with neutralising antibodies to TGFß-1 and TGFß-2. By comparison, wounds treated with TGFß-1 or TGFß-2 showed a vastly increased quantity of fibronectin in the wound at 7 days and this fibronectin had an abnormal orientation, compared to the surrounding tissue. The

same was true at 14 days, but by 6 weeks there was little difference between the TGFß-1 or TGFß-2 treated wounds and the control in terms of the quantity of fibronectin present.

At 7 days TGFß-1 treated and TGFß-2 treated and control wounds showed similar profiles of macrophage and monocyte infiltration (for example control 159, TGFß-1 149, control 117, TGFß-2 112 per section). However, TGFß-3 treated wounds had a low profile of macrophage plus monocyte infiltration (control 130, TGFß-3 91 per section).

At 7 days TGFß-1 treated and TGFß-2 treated wounds had a higher profile of macrophages in the lower half of the wounds compared to similar areas in the control wounds (control 50/TGFß-1 80, control 45/ TGFß-2 59 per section). However, in the upper half of the wounds the macrophage infiltration was similar in the TGFß-1 treated and control wounds (control 37, TGFß-1 39 per section) whilst TGFß-2 treated wounds had a lower profile (control 34, TGFß-2 19). By contrast, TGFß-3 treated wounds showed a lower macrophage profile throughout the entire wound, compared to the control wounds (upper half control 41, TGFß-3 16; lower half control 72, TGFß-3 28 per section).

Laminin staining was used as a marker of neovascularisation. At 7 days, TGFß-1 treated wounds showed an increase in the number of blood vessels, particularly at the base of the wound. TGFß-2 treated wounds appeared similar to the control wounds. TGFß-3 treated wounds, however, had many more blood vessels compared to either the control or the TGFß-1 or the TGFß-2 treated wounds. This was a very marked effect.

By 14 days there were few differences in the number of blood vessels between either the TGFß-1, TGFß-2 or TGFß-3 treated wounds compared to the control. However, the TGFß-3 treated wounds tended to have more blood vessels.

In terms of collagen deposition within the wound, as assayed by Mallory staining or immunocyto-chemistry, treatment of the wound with either TGFß-1 or TGFß-2 increased the amount of collagen within the wound on days 7 and 14 after wounding. Furthermore, this collagen had an abnormal orientation with a much higher percentage of fibres orientated in a vertical direction, compared to the surrounding dermis. At six weeks, the control, TGFß-1 and TGFß-2 treated wounds were visibly scarred with an abnormal accumulation of abnormally orientated collagen within the wounded area. By contrast, wounds treated with TGFß-3 showed slightly

less collagen deposition on days 7 and 14 after wounding. Moreover, the collagen deposited was in a similar reticular pattern to that of the surrounding dermis. Consequently, by six weeks after wounding, the TGFß-3 treated wounds had a more similar dermal architecture to that of the surrounding normal skin, compared to either the control TGFß-1 or TGFß-2 treated wounds. This result with TGFß-3 is very similar to that obtained with neutralising antibodies to TGFß-1 and TGFß-2.

In summary, therefore, treatment of the wounds with TGFß-3 decreased the amount of extracellular matrix deposited in the early wound, assured that the orientation of this matrix was in the normal reticular pattern of the dermis, compared to the abnormal pattern of the scar, decreased the number of macrophages and monocytes and hence inflammatory infiltrate into the wound, but greatly increased the number of blood vessels in the early healing wound. These effects are almost identical to those observed with neutralising antibodies to TGFß-1 and TGFß-2 except the increase in the number of blood vessels. Treatment of the wounds with neutralising antibodies to TGFß-1 and TGFß-2 decrease the amount of extracellular matrix deposited, alter the orientation of this matrix, so that it is in a more normal alignment, decrease the inflammatory infiltrate

of macrophages and monocytes (like TGFß-3) but decrease the number of blood vessels (unlike treatment with TGFß-3 which increases the number of blood vessels).

TGFß-3 therefore acts as an anti-scarring (anti-fibrotic) agent. It is very clear that this is an isoform specific effect within the TGFß family.

TGFß-3 therefore becomes a target as an anti-fibrotic agent or an anti-scarring agent. It may be capable of biological modification to increase the anti-fibrotic effect or define more carefully that portion of the molecule responsible for these effects. It may be possible to optimise the structure of TGFß-3 as an anti-fibrotic agent, based on such analysis. The effects of TGFß-3 in this regard are unpredictable from the literature, and interestingly, differ from the neutralising antibody experiments, particularly in the increase in angiogenesis. This may actually be beneficial for certain kinds of wound healing, e.g. chronic wounds such as venous leg ulcers, where one wants to increase the vascular supply to stimulate healing but decrease subsequent scarring.

In the context of fibrosis, the effects of TGFß-3 or anti TGFß-1/TGFß-2 agents are not limited to preventing further increases of fibrosis. TGFß-1/TGFß-2

act to increase the accumulation of extracellular matrix molecules both by stimulating synthesis of new extracellular matrix molecules and decreasing the removal of existing matrix molecules, i.e. inhibiting tissue turnover (Roberts and Sporn, the transforming growth factor - ß's, In: Peptide growth factors and their receptors, Springer Verlag, Berlin, 1990, p 418-472). Therefore, any agent which antagonises or neutralises or renders ineffective TGFß-1/TGFß-2 not only decreases extracellular matrix synthesis but also increases remodelling. As an anti-fibrotic agent either TGFß-3 or anti-TGFß-1/anti- TGFß-2/anti-PDGF (or some combination thereof) may in certain fibrotic diseases, e.g. glomerulonephritis, pulmonary fibrosis, reverse the accumulation of fibrous scar tissue already present in the tissue.

It will be appreciated that it is not intended to limit the invention to the above examples only, many variations, such as might readily occur to one skilled in the art, being possible, without departing from the scope thereof as defined in the appended claims.

Thus for example, as well as applying a preparation to a wound containing TGFß-3 only, this may be given in combination with fibrotic growth factor neutralising agent(s), for example, anti-TGFß-1 and/or

anti-TGFß-2 and/or anti-PDGF antibodies, in a ratio which will enable the required amount of vascularisation for the particular type of wound to be provided whilst at the same time healing the wound without scarring.

CLAIMS

1. A healing composition containing at least one non-fibrotic growth factor in combination with a pharmaceutically acceptable carrier.

2. A composition according to claim 1, wherein the non-fibrotic growth factor comprises TGFß-3.

3. A composition according to claim 1 or claim 2, wherein the non-fibrotic growth factor comprises FGF.

4. A composition according to any preceding claim, comprising anti-fibrotic agents.

5. A composition according to claim 4, wherein the anti-fibrotic agents include antibodies to TGFß-1, TGFß-2 and PDGF; binding proteins which prevent TGFß-1, TGFß-2 and PDGF from binding to their receptors by either binding to the growth factor itself, eg. Decorin, Biglycan, or binding to the receptor, eg. peptides containing the receptor binding site sequence; or soluble forms of growth factor receptor or the growth factor binding domains of these receptors or antisense oligonucleotides or ribosymes which act to prevent fibrotic growth factor mRNA translation.

6. A composition according to any preceding claim wherein the non-fibrotic growth factor and/or anti-fibrotic agent(s) are present in the composition in an active form.

7. A composition according to any of claims 1 to 5, wherein the non-fibrotic growth factor and/or anti-fibrotic agent(s) are present in the composition in an inactive form.

8. A composition according to claim 7, wherein inactivation is by encapsulation.

9. A composition according to claim 8, wherein the capsules are degradeable by an external stimulus to release the active form when required.

10. A composition according to claim 9, wherein the external stimulus includes UV light, ultrasound, in vivo enzymes or heat.

11. A composition according to claim 7, wherein inactivation is by the molecular addition of a binding molecule which is detachable when required by an external stimulus including UV light, ultrasound, in vivo enzymes or heat.

12. A composition according to any preceding claim, wherein the non-fibrotic growth factor is present in an inactive form, for example, as a precursor, and is activated upon contact with tissue containing the natural cleavage enzymes required to convert the precursor into its active form.

13. A composition according to claim 1, wherein the carrier comprises a neutral sterile cream, gel, aerosol or powder for topical application.

14. A composition according to claim 1, wherein the carrier comprises a patch or a sterile dressing or an absorbable dressing for topically covering a wound.

15. A composition according to claim 1, wherein the carrier comprises a sterile solution for irrigation, injection or inhalation.

16. A composition according to claim 1, wherein the carrier comprises a tablet, capsule, and the like, for enteral administration.

17. A composition according to claim 1, wherein the carrier comprises a biopolymer, for example collagen, hyaluronic acid or polymer, for contacting or implanting into the wound/fibrotic lesion so as to allow release of

the active agents slowly or quickly and for to be active in situ.

18. A method of preparation of a pharmaceutical healing or anti-fibrotic composition containing at least one non-fibrotic growth factor for topical application in a cream, gel, powder, aerosol, patch or dressing, biopolymer or polymer implant, delay or slow release system or in a solution for irrigation, injection or inhalation, or in a tablet or capsule for enteral administration.

19. A method of inhibiting fibrosis during the healing of wounds and other fibrotic diseases, disorders or conditions, comprising administering to a host suffering from tissue wounding or these fibrotic conditions, at least one non-fibrotic growth factor.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 93/00586

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K37/02; A61K39/395; C07K15/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K ; C07K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X,P	WO,A,9 217 206 (THE VICTORIA UNIVERSITY OF MANCHESTER) 15 October 1992 see page 4, line 11 - page 13, line 23 ---	1,3,4-19
X	WO,A,9 003 810 (ED GEISTLICH SÖHNE AG FÜR CHEMISCHE INDUSTRIE) 19 April 1990 see page 1, line 1 - line 19 ---	1,3
X	EP,A,0 375 127 (GENENTECH) 27 June 1990 see column 5, line 41 - line 51 see column 7, line 21 - column 12, line 16 ---	1,2,6-19

-/--

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
14 JULY 1993	[illegible]
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	REMPP G.L.E.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	WO,A,9 110 727 (LA JOLLA CANCER RESEARCH FOUNDATION) 25 July 1991 see page 10, line 25 - page 11, line 11 see page 22, line 6 - page 23, line 21	1,4,5
X	EP,A,0 433 225 (CIBA-GEIGY AG) 19 June 1991 cited in the application see page 4, line 29 - page 5, line 19 see page 8, line 22 - page 9, line 39	1,2,6, 13-19

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

GB 9300586
SA 72604

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 14/07/93

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9217206	15-10-92	AU-A-	1436892	02-11-92
WO-A-9003810	19-04-90	None		
EP-A-0375127	27-06-90	AU-A-	4524889	28-05-90
		CA-A-	2002130	02-05-90
		WO-A-	9004974	17-05-90
WO-A-9110727	25-07-91	None		
EP-A-0433225	19-06-91	AU-A-	6701890	13-06-91
		JP-A-	3191791	21-08-91

INTERNATIONAL SEARCH REPORT

rnational application No.
PCT/GB93/00586

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 19 is directed to a method of treatment of the humsn/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.